07. Evaluating the effectiveness of a sodium butyrate feed additive for the control of *Salmonella* carriage in finishing pigs.

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Abstract

This study aimed to investigate the effectiveness of commercially available sodium butyrate to control the shedding of *Salmonella* on two Irish pig farms with a history of high *Salmonella* seroprevalence. On both farms, pens (12-17 pigs/pen) were randomly assigned to a control (finisher feed without additives) or an acid treatment (the same feed supplemented with 0.03% sodium butyrate) for 24-26 days prior to slaughter. On Farm A, *Salmonella* shedding was reduced in the acid group compared to the control group at the end of the treatment period (30% vs. 57% probability of detecting *Salmonella* in faeces, respectively; $p<0.05$). However no effect of treatment was observed on Farm B, which could perhaps be explained by a concomitant infection by *Lawsonia intracellularis*. No significant differences in *Salmonella* recovery rates were observed from caecal digesta or ileocaecal/mesenteric lymph nodes collected at slaughter in either of the trials. Furthermore, feed intake, weight gain and feed conversion efficiency did not differ significantly between control and treatment groups on either farm.

Introduction

Ireland has a high prevalence of *Salmonella* contamination on pork carcasses (20%) (EFSA, 2008) and this is likely related to a high level of *Salmonella* carriage in some pig herds within the country (Burns *et al.*, 2013). This highlighted a need to find measures to control *Salmonella* shedding in pigs at primary production, especially in finishing pigs, as they are a significant source of *Salmonella* in the abattoir. Dietary supplementation with organic acids has shown promise in controlling *Salmonella* in pigs (Friendship *et al.*, 2006). After a critical review of the literature, sodium butyrate was selected for further evaluation. Sodium butyrate acts via down-regulation of the expression of *hil*A (an invasion gene) in *Salmonella*, thereby suppressing its ability to invade porcine intestinal epithelial cells, which in-turn decreases faecal shedding of the bacterium in pigs (Boyen *et al.*, 2008). This study investigated the ability of dietary supplementation with sodium butyrate during the last month of growth pre-slaughter to reduce faecal shedding and intestinal carriage of *Salmonella* in finisher pigs.

Material and Methods

(a) Farms

Farm A had a historically high *Salmonella* seroprevalence (according to the Irish National Pig *Salmonella* Control Programme) i.e. > 60%; however, prior to commencement of the trial, the seroprevalence declined to 0%. Thus, artificial contamination of pens using a *Salmonella* strain recovered from sows on the farm during the same time period was performed in advance of the feeding study. This strain was a monophasic variant of *S.* Typhimurium (4,5,12;i:-), and its antimicrobial resistance pattern was determined to be ASSuT. Farm B, on which pigs had been shown to carry *S.* Typhimurium, also had a historically high *Salmonella* seroprevalence (i.e. > 50%).

(b) Animal Housing and Diets

Farm A had 14 pens with a total of 168 pigs (72 males and 96 females, with 12 same gender pigs/pen),

while Farm B had 12 pens with a total of 177 pigs (86 males and 91 females, with 12-17 same gender pigs/ pen). Approximately 4-weeks before the target slaughter date, pigs on both farms were randomly assigned to one of two dietary groups: a standard finisher feed with no feed additive (control) or the same finisher feed with 0.3% sodium butyrate (Adimix®, Nutriad, Kasterlee, Belgium; acid). The sodium butyrate additive used was coated to ensure delivery to the lower intestinal tract.

(c) Sample Collection

On both farms, pigs were weighed before diets were administered (day 0) and before pigs were sent to slaughter (day 26 for Farm A and day 24 for Farm B). Feed intake for each pen was recorded on a weekly basis. Faecal samples (25 g) were collected from each pig on days 1, 12 and 24/26; and swabs were taken from the trucks used to transport pigs to the abattoir (before loading) and from two holding pens at the abattoir. On day 28, all 168 pigs from Farm A were sent to slaughter; whereas for Farm B, 88/177 pigs were sent to slaughter on day 26 and the remaining 89 pigs were sent to slaughter on day 32. Following slaughter, caecal digesta (≥ 25 g), and ileocaecal lymph nodes (ILN) together with mesenteric lymph nodes (MLN) (≥ 10 g) were collected from 88 animals (45 control and 43 acid) from each farm for *Salmonella* detection. The ILN and MLN were pooled prior to analysis. Carcass quality data were also collected from each animal post-slaughter. All samples were collected aseptically and kept cool on ice until analysis (within 24 hours).

(d) Sample Analysis

Salmonella Detection and Serotyping: The presence/absence of *Salmonella* was determined in all samples using standard enrichment procedures (ISO 6579:2007; Amendment 1: Annex D). Serotyping of all presumptive *Salmonella* isolates was performed using a real-time PCR assay for identification and differentiation of *Salmonella* enterica serovar Typhimurium and monophasic serovar 4,[5],12:i:- as described by Prendergast *et al.* (2013). Any isolates not identified as *Salmonella* Typhimurium or its monophasic variant, were serotyped according to the White-Kauffmann-Le Minor scheme using commercial antisera.

Statistics: All statistical analysis was performed using the Proc Glimmix procedure of SAS (SAS Inc, Cary, N. Carolina).

Results

On Farm A, after 26 days, administration of sodium butyrate resulted in a decline in *Salmonella* shedding as compared to the control group (30% vs. 57% probability of detecting *Salmonella* in faeces, respectively; $p<0.01$; Figure 1a). When comparing the effect of treatment between days 12 and 26, supplementation with sodium butyrate was again more effective in reducing *Salmonella* shedding (66% vs. 30% probability of detecting *Salmonella* in faeces, respectively $p<0.001$) than no supplementation (50% vs. 57%, respectively, $p=0.80$, Figure 1a). No effect of acid treatment on *Salmonella* shedding was observed for Farm B (Figure 1b). Truck swabs for both farms were *Salmonella*-negative; while the 2 lairage pens swabbed (for Farm B) were *Salmonella*-positive.

No significant treatment differences in *Salmonella* recovery rates were observed in the caeca or ILN/ MLN collected at the slaughterhouse for either farm (Figure 1c). The *Salmonella* serovars recovered from the faecal samples were *S.* 4,5,12:i:- (Farm A) and *S.* Typhimurium 1,4,[5],12:i:1,2 (Farm B). The caecal and ILN/ MLN isolates were serotyped as *S.* 4,[5],12:i:- (Farm A and B) and *S.* Typhimurium 1,4,[5],12:i:1,2 (Farm B) and

the lairage isolates as *S.* 4,[5],12:i:-.

In terms of production parameters, no significant differences were observed in feed intake, weight gain or feed conversion efficiency (FCE) between control and acid-treated groups for both farms. For Farm B, female pigs on the acid treatment had a significantly higher kill-out yield than male pigs on the acid treatment (79.4% vs. 77.1%, respectively; p=0.02). There was also a tendency for females to have a higher kill-out yield than males (79.0% vs. 78.0%; p=0.06). Muscle depth of pigs in the control and acid-treated groups were 51.8 mm and 50.1 mm, respectively (p=0.06).

Discussion

This study evaluated the effectiveness of a commercially available sodium butyrate in reducing *Salmonella* shedding and intestinal carriage in finisher pigs and also investigated its ability to improve growth performance. Dietary supplementation with sodium butyrate was successful in decreasing *Salmonella* shedding over a 26-day period on a highly contaminated farm with no secondary infection. This result is in line with previous research that showed reductions in *Salmonella* shedding in weaner pigs deliberately infected with *Salmonella* after 12 days of dietary supplementation with sodium butyrate (Boyen *et al.*, 2008). However, this is the first on-farm trial that has used sodium butyrate as a control measure to reduce the shedding of *Salmonella* in finishing pigs. A co-infection by *Lawsonia intracellularis* on Farm B may explain the absence of a sodium butyrate effect on that farm. In addition, for Farm B, the caecal and ILN/MLN isolates were identified as both *S.* Typhimurium and its monophasic variant. The latter serotype was not recovered from the pig faeces at farm level but was recovered from the two lairage pens sampled at the abattoir, prior to loading the pigs. This suggests that the pigs from Farm B may have also acquired a new infection in the lairage.

Despite the absence of statistically significant effects on growth, pigs fed the acid treatment on Farm A showed a 7% increase in growth rate and an 8% improvement in FCE over the 26-day feeding period compared with pigs on the control diet. Similarly, for Farm B, pigs on the acid treatment showed a 2.6% increase in growth rate and a 4% improvement in FCE over the 24-day feeding period compared with those on the control diet.

Conclusion

Overall, dietary supplementation with sodium butyrate for a relatively short period of time (<30 days) prior to slaughter can be considered an effective control measure to reduce faecal shedding of *Salmonella* in finishing pigs. However, it did not reduce intestinal carriage at slaughter and did not significantly improve growth performance in pigs.

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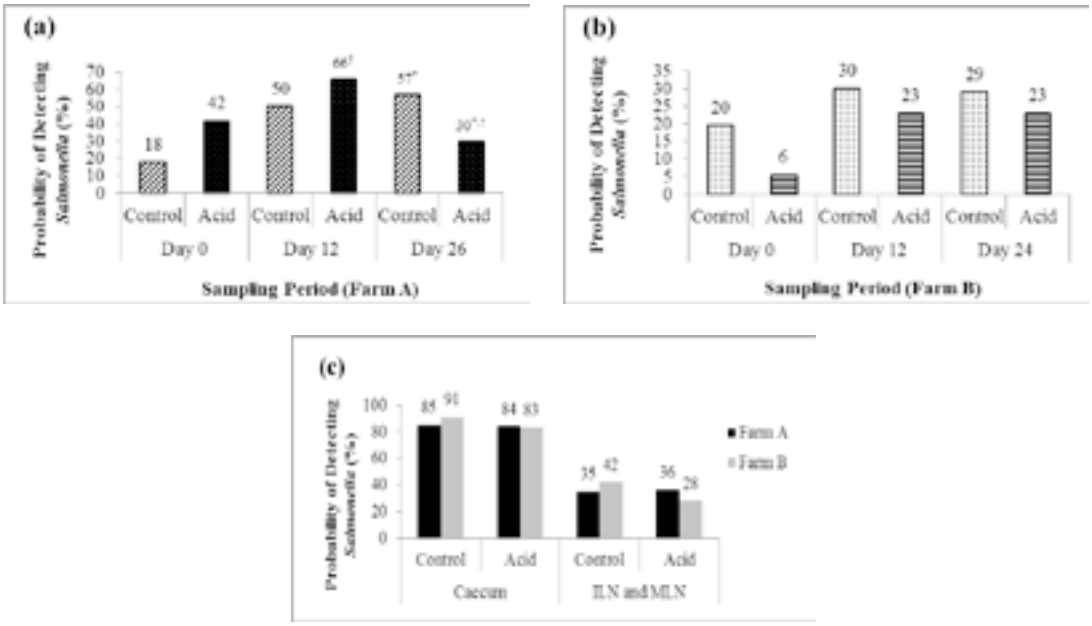
Table 1. *Salmonella* prevalence in faeces collected from individual finisher pigs fed either a control diet or a diet supplemented with sodium butyrate during sampling days 0, 12, 24/26 for Farms A and B

	Farm A						Farm B					
	Day 0		Day 12		Day 26		Day 0		Day 12		Day 24	
	Control	Acid	Control	Acid	Control	Acid	Control	Acid	Control	Acid	Control	Acid
No. Pigs with Positive Faeces	15	35	43	53	47	28	17	5	27	20	28	20
No. Pigs with Negative Faeces	65	44	40	28	34	50	66	83	55	67	58	68
No. Pigs with No Faeces	5	5	2	3	4	6	4	2	5	3	1	2
Total Pigs	85	84	85	84	85	84	87	90	87	90	87	90
Salmonella Prevalence	17.65	41.67	50.59	63.10	55.29	33.33	19.54	5.56	31.03	22.22	32.18	22.22



*p<0.001 (acid group on day 12 versus day 26)
†p=0.018 (control group versus acid group on day 26)

Figure 1. The probability of detecting ***Salmonella*** in faeces (a, b), caeca and ILN/MLN (c) from finisher pigs in control versus acid treatment groups for Farms A and B